A Pre-synopsis entitled as

"Microbiological Control of *Fusarium Wilt* of Tomato (*Lycopersicon esculentum* Mill.)"

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Submitted to

Swami Ramanand Teerth Marathwada University
Nanded

For the Degree of

Doctor of Philosophy in Microbiology

Under the Faculty of Science and Technology

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1. Introduction:

Tomato (*Lycopersicon esculentum* Mill) is an important and widely grown vegetable crop around the world and it belong to the family of Solanaceae. It ranks second in world among the vegetables, only after potato. According to Srivastava *et al.,* (2019) Tomato is referred as a "poor man's orange" because of its attractive appearance and nutritive value. Tomatoes are part of healthy and proportional balanced diet of Human beings. They are rich in essential amino acids, vitamins, minerals, iron, phosphorus and sugars (Burton & Reimers, 2011). Tomato fruit can be consumed in both fresh and processed forms. Red tomatoes may contribute protection to human health as it consists of an antioxidant and a carcinogenic substance (Sun *et al.,* 2015). Tomatoes are composed of important constituents for bone health, neurological diseases and anticancer benefits. (Deepti, 2016.)

China is the world's largest tomato producer with 64,768.16 million kilos, i.e., 34.67% of the world's total production. In 2020, China devoted 1,107,485 hectares to tomato production and its yield per square meter stood at 5.85 kg/m². China was followed by India, which in 2020 produced 20,573 million kilos of tomatoes on 812,000 hectares and had an average yield of 2.53 kg/m² (https://www.hortidaily.com/article/9387133/worldwide-tomato-production-exceeds-186-800-million-kilos/).

The major Tomato producing states in India are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Bihar, Telangana, Tamil Nadu, Uttar Pradesh, Maharashtra, Haryana and Himachal Pradesh. These states are account for about 90% of the total production of the country (http://www.nhb.gov.in/statistics/Reports/Tomato-for-October-2019.pdf).

As per the report by Horticulturists Statistics Division, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Govt. of India, New Delhi (2019), in the year 2017-18, the share of Tomato production of Maharashtra state was 5.50% and in the year 2018-19 the share of tomato production found to be reduced up to 4.15%. This reduction in the share of Tomato production in Maharashtra could be occurred due to the various factors like attack by several phytopathogens affecting tomato plant use of agrochemicals etc.

Among the major diseases affecting tomato plant in India mostly belongs to the fungal and bacterial pathogens. In India also *Fusarium* Wilt is a major threat to tomato production that has been reported to cause 25-55% productivity loss in different parts of the country (Nirmaladevi *et al.,* 2016). *Fusarium* Wilt is characterized by drooping leaves followed by chlorosis, necrosis, browning or discoloration of vascular tissues finally leading to the death of the plant. The

three pathogenic races of Fol have been identified depending upon pathogenicity on tomato cultivars (Nirmaladevi *et al.,* 2016).

Fusarium Wilt is troublesome and difficult to control once established in soil (Haridon *et al.,* 2011). On other hand, technically it is difficult and expensive to treat large amount of soil with chemical. No effective chemical control measures have been devised for *Fusarium* till date although resistant cultivars have provided some extent of control but indeed no commercially acceptable tomato cultivar is available with adequate resistance (Jones *et al.,* 1991). The continuing problem of greater chances of emergence of new races in Fusarium has urged to look for a promising management alternative (Borrero *et al.,* 2006). In above context, certain rhizospheric isolates have emerged as a safer and eco-friendly alternative to chemical pesticides.

***Fusarium* Wilt of Tomato** *(Lycopersicon esculentum* Mill*)*:

Tomato crop throughout the world is affected by *Fusarium* species causing soil-borne diseases of great economic importance. *Fusarium* Wilt of tomato caused by *Fusarium oxysporum f.* sp. *lycopersici* (Sacc.) Snyder and Hans lead to high losses of tomatoes in many countries. *Fusarium oxysporum f.* sp. *lycopersici* is a soil-borne in nature and is invades plants through wounds on roots. Infected plants become stunted, chlorotic and Wilt (Jones, 1991; McGovern, 2015).

Wilting is a result of impaired water translocation due to clogging of vessels by mycelium, spores, gels, gums and tylosis. The fungus also produces toxins such as fusaric acid and lycoparasmin which chelate with iron and cause iron deficiency in plants and consequently yellowing of leaves (Gäumann, 1951).

Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios, 1988). The resulting spores can then be used as new inoculum for further spread of the fungus.

The control of this pathogen is difficult due to the survival of its resting structures (Chlamydospores) in the soil for many years without a host and due to its progress within vascular tissues (Ignjatov *et al.,* 2015). Moreover, chemical and genetic control of tomato *Fusarium* Wilt failed to successfully suppress the disease due to the risk of fungicide resistance development and to the emergence of new physiological races of *FOL* (Ge *et al.,* 2004). Therefore, research studies have been more focused on the eco-friendly alternatives for efficient pathogen control (Reis *et al.,* 2004).

Practically, *Fusarium* Wilt of tomato can be adequately controlled by applying systemic fungicides like Bavistin [Carbendazim], Benlate [Benomyl], Thiram + Bavistin, Roko (Thiophanate-methyl), Thiram, Agrosan G.N. [Phenylmercury acetate], Captan, Vitavax [Carboxin], Companion, Mancozeb and Ridomil [Metalaxyl] However, these fungicides are costlier and are very harmful to humans and the environment. In addition, the excess use of these fungicides has developed resistance in *Fusarium* Wilt Phytopathogen. also, these systemic fungicides affecting the natural microflora near the rhizosphere of healthy plant. Therefore, in order to cope up this problem we are going to search for effective microbial control agent against *Fusarium* Wilt Phytopathogen of tomato which will be ecofriendly and sustainable for Tomato crop. (Kishore & Singh, 2008).

2. Review of Literature and Development in the subject:

The present research is planned for searching of effective microbial control agents against *Fusarium* Wilt of tomato crop which will be ecofriendly for environment and for sustainable agriculture. Sufficient literature is available on biocontrol of *Fusarium* Wilt of tomato which is represented as follows:

Different fungal microbial control agents such as *Aspergillus spp.,* (El-Sayed *et al.,* 2020; Ngo *et al.,* 2021) *Ampelomyces sp.,* (Carbó *et al.,* 2020; Huth *et al.,* 2021) *Candida* sp., (Carbó *et al.,* 2018; Sui *et al.,* 2020) *Gliocladium sp.,* (Rashad and Moussa, 2020; Hassine *et al.,* 2022) and *Trichoderma spp.* (Sood *et al.,* 2020; Poveda, 2021.Ferreira and Musumeci, 2021) are in use to control various phytopathogens.

Several authors have been reported that various bacterial biocontrol agents such as *Bacillus spp.*, *Pseudomonas spp.*, *Streptomyces spp., Burkholderia spp.* (Islam *et al.,* 2019; Theradimani *et al.,* (2018) to control the phytopathogens.

Muhammad *et al.,* (2013) reported *Trichoderma harzianum* was the most effective antagonists in reducing number of wilted plants with 67.93 percent reduction in disease incidence followed by *Pseudomonas fluorescens, Penicillium spp.* and *Azotobacter spp.* with 55.21%, 50.69% and 48.96 %, while *Rhizobium spp.* was least effective one with only 34.38 percent reduction in disease incidence.

Juber *et al.,* (2014) evaluated the efficiency of biocontrol agents, *Bacillus mycoides* and *Trichoderma* viride and the chemical compounds, ASM and Preservepro against *Fusarium oxysporum* f.sp. *lycopersci* the causal agent of tomato wilting disease in culture media and under greenhouse conditions. The results showed that all the control agents exhibited an inhibition rate on *F. oxysporum* growth on both culture media and under natural conditions.

Kumar, (2019) reported that *Trichoderma harzianum* was found inhibiting fungal mycelial growth in vitro radial growth of *F. oxysporum* f.sp. *lycopersici* (61.34%) and disease incidence (29.67%) under natural conditions in vivo. Tomato plants showed an increased plant height (69.53 cm) and the root length (by 55.68 cm) when compared with control (63.86 cm and 49.43 cm respectively for plant height and root length).

Bhattacharya *et al.,* (2019) reported that six antagonistic bacterial strains were screened against *Fusarium oxysporum* from 344 isolates. Out of these six antagonistic bacterial endophytes, *Bacillus tequilensis* (PBE1) (MTCC25188) was proven most effective and an eco-friendly substitute of chemical fungicides against *Fusarium oxysporum* for tomato Wilt disease management.

Kamali *et al.,* (2019) reported that 303 *Bacillus spp.* isolates were obtained from tomato rhizospheric soil and screened for antagonism against *Fol.* Among them, 20 isolates were found to inhibit pathogen growth by 67.77% and 33.33% in dual culture and volatile metabolite tests, respectively.

Ramírez *et al.,* (2021) evaluated the ability of *B. cereus* MH778713, a strain isolated from root nodules of *Prosopis laevigata*, to protect tomato plants against *Fusarium* wilt.

Devi *et al.,* (2022) evaluated the endophytic *Bacillus* isolates ERBS51 (*Bacillus velezensis*) and (*Bacillus spp.*) and ERBS10 showed a maximum inhibition against *FOL*, with 58.43 and 55.68%, respectively, in a dual culture experiment.

3. Objectives of research/proposed hypothesis:

Considering the importance of tomato in our day to-day life and health of human beings and the influence of phytopathogens on their productivity, following objectives set to resolve the problem of major *Fusarium* Wilt of Tomato by using rhizospheric isolates and study in detail the biocontrol abilities of these rhizospheric isolates against *Fusarium* phytopathogens of Tomato crop in a cost-effective and eco-friendly manner.

The microorganisms residing in the vicinity of plant roots are termed as Rhizospheric microorganisms. These rhizospheric microorganisms may be associative, mutualistic, competitive or antagonistic (Jain *et al.,* 2020), resulting in neutral, beneficial as well as the detrimental effect on plants.

The beneficial rhizospheric microorganisms combat with phytopathogens either by inhibiting or suppressing their growth by the deployment of an array of mechanisms including competition for nutrition and space, mycoparasitism, antibiosis and inducing systemic resistance (ISR) either individually or simultaneously (Babu *et al.,* 2015; Patel and Saraf, 2017).

Several rhizospheric bacteria and fungi such as species belonging to genera *Bacillus, Pseudomonas, Trichoderma*, Arbuscular mycorrhizal fungi (AMF) utilize one or more of these biocontrol mechanisms for management of phytopathogens and tailoring abiotic stress that ultimately leads to enhanced plant productivity (Babu *et al.,* 2015; Kumar *et al.,* 2017).

Biological control methods have the advantage of being non-toxic to the environment. It is an innovative, cost effective and eco-friendly approach. The present research work will be conducted to isolate native rhizospheric microbial isolates possessing the ability to manage *Fusarium* Wilt of tomato crop plant.

The objectives are as follows:

1. Isolation of *Fusarium* Wilt phytopathogen from infected stem of Tomato plant and its pathogenicity testing.
2. Isolation of rhizospheric microorganisms from healthy plant.
3. Screening of rhizospheric isolates against *Fusarium* Wilt phytopathogens for microbiological control ability.
4. Identification of effective rhizospheric isolates.
5. Deciphering of mechanism of rhizospheric isolates.
6. Pot assay for microbiological control ability of rhizospheric isolates against phytopathogens.
7. Pot assay for growth promotion of tomato by using the efficient rhizospheric isolates.

4. Methodology:

In order to investigate the problem following methodology will be adopted:

4.1. Isolation of *Fusarium* Wilt phytopathogen from infected stem of Tomato plant.

Isolation of *Fusarium* Wilt phytopathogen carried out by using the method of Raut and Hamde., (2016). *Fusarium* Wilt phytopathogen was isolated from infected stem of tomato plant using potato dextrose Agar (PDA) plates and growth of fungal mycelium then transferred on fresh PDA and purified based on macro and microscopic observations of mycelial growth and will be identified as species *Fusarium oxysporum* (Watanabe, 2010) The phytopathogen then transferred on fresh PDA and Kings B agar plates and also on maintained on slants for further use.

4.2 Isolation of rhizospheric microorganisms from healthy plant:

Isolation of rhizospheric microorganisms is caried out by serial dilution technique from rhizosperic soil of different healthy plants.

4.3 Screening of rhizospheric isolates against *Fusarium* Wilt phytopathogens for microbiological control ability.

All the rhizospheric isolates will be screened for antagonistic activity against *Fusarium* Wilt Phytopathogens of Tomato by using Dual Culture Technique (Rakh *et al.,* 2011).

4.4 Identification of effective rhizospheric isolates.

Effective rhizospheric isolates identified by rRNA sequencing method.

4.5 Deciphering of mechanism of rhizospheric isolates.

To characterize the mechanism of microbiological control agent, the efficient rhizospheric isolates were tested for the production of volatile metabolites, diffusible metabolites, siderophore etc. (Raut and Hamde., 2016).

4.6 Pot assay for microbiological control ability of rhizospheric isolates against phytopathogens.

The Pot Assay for the microbiological control ability of effective rhizospheric isolates is carried out to confirm the result of plate assay in lab to land according to John *et al.,* (2010).

4.7 Pot assay for growth promotion of tomato by using the efficient rhizospheric isolates

The Pot Assay for the microbiological growth promoting ability of effective rhizospheric isolates is carried out as per John *et al.,* (2010).

5. Importance of the study:

Wilts induced by *Fusarium oxysporum* are difficult to control due to multihostile nature and presence of chlamydospores soil borne habitat. by using conventional chemical fungicides. Present day agriculture technology promotes the application of large amount of systemic fungicides to obtain maximum productivity which causes ecological imbalance and health hazards.

The indiscriminate use of chemical control agents results in environmental pollution and in the increase of chemical toxicity in nature's food chain. Since the effect of most fungicides are temporary, they had to applied continuously which in turn results in ecological imbalance (Zubrod *et al.,* 2019). The use of volatile chemicals against pests and pathogen control may lead to the depletion of ozone layer. Moreover, development of resistance in a pathogen population due to constant exposure to these chemicals is also undesirable chemical fumigation of the soil pollutes the ground water which is hazardous to the consumer and wild life (Ziedan, 2022). The high cost of fungicides causes socio-economic problem among resource poor farmers. To overcome these hardships several attempts have been made to control plant diseases by biological means.

The recent update of agricultural field, it has become crystal clear that tomato is one of the most important vegetable crops for the health of people because of its nutritive value. Hence it is essential to improve the yield both quality and quantity wise to satisfy the demands of ever-increasing population. In this context variety of synthetic agrochemicals are used by farmers to increase

the yield of tomato crop and to control the phytopathogens of tomato. This practice has led to many more environmental problems like i) Disturbance of ecological balance of soil, ii) contamination of ground water. iii) Development of resistance among the pathogens towards the synthesis chemicals. iv) Severe health risk to non-target species like humans etc.

Microbiological control using rhizospheric microorganisms is a newly developing technology for the management of phytopathogens of crops. To cope up with these issues an attempt has been made through this research work by using a target specific, rhizospheric isolates for the efficient control of *Fusarium* Wilt phytopathogen of tomato in an ecofriendly and cost-effective manner.

5.1 International status:

Microbes from rhizospheric niches have great potential as a source of microbiological control agent for plant pathogens. The presence of root exudates has a pronounced effect on the rhizospheric microorganisms where they serve as an energy source, promoting growth and influencing the root system for the rhizobacteria. In certain instances, they produce secondary metabolites which inhibits the growth of soil-borne phytopathogens for the advantage of the plant. Several authors have reported the use of rhizosperic microorganisms as potential control measures for the phytopathogens:

Sallam *et al.,* (2019) Seven isolates of *Trichoderma spp*. evaluated *in vitro* by bioassay for their potential to antagonize *Fusarium oxysporum* f.sp. *lycopersici* (the causal pathogen of tomato wilt disease). The highest percentage of inhibition against the tested pathogenic isolate were obtained with *T. atroviride* and *T. longibrachiatum* isolate. the highly antagonistic isolates of *T. atroviride* and *T. longibrachiatum* led to a significant decrease of disease severity compared to the untreated control treatment. The lowest severity was achieved with the *T. longibrachiatum* isolate (24.8%) followed by isolate *T. atroviride* (34.6%) compared with the other tested isolates.

In a continued effort to discover microorganisms with plant protective properties, Doan *et al.,* (2020) isolated and screened bacterial isolates from the genus *Collimonas*, for their ability to inhibit in vitro the growth of *Fusarium oxysporum* f. sp. *lycopersici.* Isolate *Collimonas arenae* Cal35 showed the greatest all-round performance against *Fusarium oxysporum* f. sp. *lycopersici.*

Vanegas *et al.,* (2020) screened 52 isolates against *Fusarium oxysporum* f. sp. *lycopersici* in dual culture technique. The isolate *S. marcescens* GIBI127 showed the best percentage of Inhibition of spore germination of *Fol* (59.29%).

Vinchira-Villarraga *et al.,* (2021) evaluated 152 marine-derived bacterial isolates for the antifungal activity against the tomato phytopathogen *Fusarium oxysporum* f. sp. *lycopersici.* Out of 152 isolates, 28 Firmicutes strains showed

antifungal activity against ten isolates of *F. oxysporum* in *in vitro* testing. From them, *Paenibacillus* sp. PNM200 was identified as a potential plant growth promoting rhizobacteria and as a biocontrol agent against *Fusarium* vascular wilt.

All these reports suggest that internationally lot of work is going on for the management of *Fusarium* Wilt of tomato.

5.2 National Status

In India, the several research is actively ongoing involving the management of yield and diseases of tomato at ICAR- Indian Institute of Vegetable Research, Varanasi. Several investigators working at the national level in India have been reported as follows:

Arya *et al.,* (2018) screened two strains of *Pseudomonas* sp. for in vitro antagonism against *Fusarium oxysporum* f.sp. *lycopersici.* They were assessed for siderophore production qualitatively and quantitatively. Strains SPs9 and SPs20 produced 59.30 and 52.74 lg ml-1 siderophore and exhibited 3.4 and 3.8 cm fungal inhibition zone respectively.

Jangir *et al.,* (2018) reported that *Bacillus* isolates found effective inhibiting the growth of *Fusarium oxysporum* f.sp. *lycopersici.* during the *in vitro* dual culture technique. The *Bacillus* strain was capable of producing hydrolytic enzymes such as β-1,3-glucanase (15.61 U ml-1 min-1), protease (1608.15 U ml-1 min-1), and chitinase (129 U ml-1 min-1), volatiles and non-volatile metabolites.

Brujal *et al.,* (2021) tested *in vitro* the biocontrol agents such as *Trichoderma* sp., *Pseudomonas* sp. and *Bacillus* sp. against *Fusarium oxysporum* f. sp*. lycopersici*. Among these biocontrol agents, the fungal bioagents *Trichoderma viride* was found to be superior as compared to remaining three species which found to inhibit 84.84 per cent growth of the pathogen followed by *T. harzianum* (72.54%), *T. hamatum* (69.93%), *T. koningii* (61.49%) inhibited growth of pathogen and among two bacterial species *B. subtilis* were found to be effective in inhibiting the growth of pathogen i.e. (79.25%) as compared to *P. fluorescens* which was found to inhibit (62.36%) growth of pathogen.

Devi *et al., (2022)* evaluated the endophytic *Bacillus* velezensis and *Bacillus sp.* against *Fusarium oxysporum* f. sp. *lycopersici*, a causal agent of tomato which showed a maximum inhibition against with 58.43 and 55.68%, respectively, in a dual culture experiment. Both isolates produced iturin A, surfactin, bacillomycin D, protease, cellulase, pectinase, alpha-amylase, siderophore, ammonia and ZnCO3 solubilization.

5.3 Significance of the study:

Significance of this research lies in the fact that tomato growers can get an alternative to chemical fungicides that being used to manage diseases of tomato including *Fusarium* Wilt of tomato. Finally, the potential Rhizospheric isolates after evaluating for microbiological control agent against *Fusarium* Wilts of Tomato will be taken for commercialization. Such potential formulation of microbiological control agent which increases disease control in one hand and yield in other with easy availability in the market could change the life of tomato growing farmers as well as agricultural sectors in eco-friendly manner. This well prepared rhizosperic isolates formulation would be applied and tested for disease control and productivity in several vegetable crops like brinjal, potato, cucumber, Bhindi etc.

6. Proposed Work Plan:

I six-months	Isolation of *Fusarium* Wilt phytopathogen from infected stem of Tomato plant.
II-six-months	Isolation of rhizospheric microorganisms from healthy plant Screening of rhizospheric isolates against *Fusarium* Wilt phytopathogens for microbiological control ability.
III-six-months	Identification of effective rhizospheric isolates. Deciphering of mechanism of rhizospheric isolates.
IV-six-months	Pot assay for microbiological control ability of rhizospheric isolates against phytopathogens.
V- Six-months	Pot assay for growth promotion of tomato by using the efficient rhizospheric isolates.

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